#9B
Entry
5-16-02

**PATENT APPLICATION**

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**





In re application of

Masato HORIE, et al.

Appln. No.: 09/787,360 Group Art Unit: 1646

Confirmation No.: 7873 Examiner: Chernyshev, O.

Filed: March 16, 2001

For: LY6H GENE

**AMENDMENT UNDER 37 C.F.R. § 1.111**

Commissioner for Patents
Washington, D.C. 20231

Sir:

This Amendment is in response to the Office Action dated December 13, 2001, for which a Petition for Two Month Extension of Time, along with payment of the appropriate fee is attached, making response due on or before May 13, 2002.

Please amend the above-identified application as follows:

**IN THE CLAIMS:**

**Please cancel claims 19 and 21 without prejudice or disclaimer.**

**Please enter the following amended claims:**

Claim 20 (amended) An antibody which binds specifically to a protein comprising an amino acid sequence of SEQ ID NO:1, or an amino acid sequence with at least 70% homology to the amino acid sequence of SEQ ID NO:1, wherein the protein exhibits at least one physiological activity selected from the group consisting of neuronal survival-supporting

activity, nerve elongating activity, nerve regenerating activity, neoroglia-activating activity and brain memory-forming activity.

Claim 23 (amended) An antibody which binds specifically to an expression product expressed by a host cell comprising an expression vector comprising a DNA molecule comprising the nucleotide sequence of SEQ ID NO:3, operably linked to a promoter.

**REMARKS**

Claims 19-23 are all the claims pending in the application. Claim 19 is non-elected and withdrawn from consideration. Claims 20-23 are under examination.

The amendment cancels claims 19 and 21. The amendment to claim 20 provides an additional structural element to the recited protein of SEQ ID NO:1. Support for this element may be found at page 10, lines 23-25 of the specification. The amendment to claim 23 further clarifies the claimed invention and specifically defines the recited expression product. Support for this amendment may be found at page 16, line 23, through page 17, line 11, of the specification.

Applicants respectfully submit that the amendments are fully supported and no new mater has been introduced, hence Applicants respectfully request entry of the same.

**Claim Rejection - 35 U.S.C. § 101**

At page 2 of the Office Action, paragraph 4, the Examiner rejects claims 20-23 under 35 U.S.C. § 101. The Examiner asserts that since the function and biological significance of LY6H protein is unknown (or not described in the specification), there is no substantial or specific

utility for antibodies which bind to the protein. The Examiner's position is that based upon the record so far submitted, Applicants have not provided enough evidence to show that the claimed antibodies have a specific, substantial and credible utility.

In response, Applicants enclose herewith a Rule 132 Declaration prepared by one of the inventors, Masahito Horie, and a copy of the reference cited therein (Namgung et al., *Brain Research*, 689: 85-92 (1995)). The Declaration makes clear the function and biological significance of the LY6H protein or the expression product to which the antibody of the present invention binds. More specifically, the Declaration shows the results of an experiment proving that the LY6H protein exhibits a brain memory-forming activity and that the level of the LY6H protein is decreased in patients with Alzheimer's disease.

The data indicate the important role of LY6H in synaptic transmission in the hippocampus and reveal that a decrease in LY6H expression causes memory loss. Further, the data indicate that the expression level of the LY6H protein in Alzheimer's patients (n=11) is reduced. As demonstrated by the data, the LY6H protein can be used as a diagnostic marker for Alzheimer's disease.

The Declaration provides substantiating evidence to support the asserted function and biological significance of the LY6H protein. Applicants submit that the antibody of the invention has a clear and credible utility in detecting the diagnostic marker LY6H. Thus, Applicants respectfully request reconsideration and withdrawal of this rejection.

**Claim Rejection - 35 U.S.C. § 112, first paragraph**

**A.** Stemming from the above rejection under § 101, the Examiner rejects, at page 5 of the Office Action, claims 20-23 under 35 U.S.C. § 112, first paragraph. The Examiner asserts that if the claimed invention is not shown to be useful, then it fails to enable others to use the invention under Section 112.

Applicants respectfully traverse this rejection. As explained above, the enclosed Rule 132 Declaration supports the utility of the claimed antibody. Thus, Applicants maintain that the specification enables claims 20-23.

Accordingly, Applicants respectfully request reconsideration and withdrawal of this rejection.

**B.** At page 6, paragraph 5, of the Office Action, the Examiner rejects claim 23 under 35 U.S.C. § 112, first paragraph. The Examiner acknowledges that Applicants describe in the specification an antibody to the protein from SEQ ID NO:1. The Examiner maintains, however, that Applicants (i) fail to teach or describe any other antibodies, and (ii) do not describe a sufficient number of species to represent the genus in claim 23.

Applicants have amended claim 23 to recite that the DNA molecule coding for the expression product comprises SEQ ID NO:3. This DNA molecule codes for the protein of SEQ ID NO:1. As stated by the Examiner, Applicants describe in the specification an antibody to the protein from SEQ ID NO:1. Thus, the specification describes the subject matter of amended claim 23.

Accordingly, Applicants respectfully request reconsideration and withdrawal of this rejection.

**Claim Rejection - 35 U.S.C. § 112, second paragraph**

At page 9 of the Office Action, paragraph 6, the Examiner rejects claim 23 under 35 U.S.C. § 112, second paragraph. The Examiner states that the recitation of "stringent hybridization conditions" does not properly describe the scope of the claim.

Applicants have amended claim 23 to delete that portion of the claim. Claim 23 properly describes the scope of the claim. Accordingly, Applicants respectfully request reconsideration and withdrawal of this rejection.

**Claim rejection under 35 U.S.C 102**

At page 10, paragraph 7, of the Office Action, the Examiner rejects claim 21 under 35 U.S.C. § 102(b) as anticipated by Armstrong et al. (Journal of Comparative Neurology 216:53-68 (1983)).

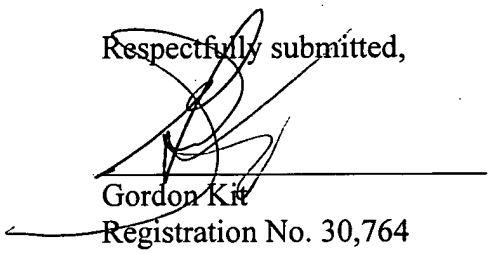
Applicants cancel claim 21, thus rendering this rejection moot. Applicants respectfully request reconsideration and withdrawal of this rejection.

**Conclusion**

In view of the above; reconsideration and allowance of this application are now believed to be in order, and such actions are hereby solicited. If any points remain in issue which the Examiner feels may be best resolved through a personal or telephone interview, the Examiner is kindly requested to contact the undersigned at the telephone number listed below.

Applicant hereby petitions for any extension of time which may be required to maintain the pendency of this case, and any required fee, except for the Issue Fee, for such extension is to be charged to Deposit Account No. 19-4880.

Respectfully submitted,



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Date: May 13, 2002

## APPENDIX

## VERSION WITH MARKINGS TO SHOW CHANGES MADE

**IN THE CLAIMS:**

**Claims 19 and 21 are canceled.**

**The claims are amended as follows:**

Claim 20 (amended) An antibody which binds specifically to a protein comprising ~~the~~ an amino acid sequence of SEQ ID NO:1, or an amino acid sequence with at least 70% homology to the amino acid sequence of SEQ ID NO:1, wherein ~~said~~ the protein exhibits at least one physiological activity selected from the group consisting of neuronal survival-supporting activity, nerve elongating activity, nerve regenerating activity, neoroglia-activating activity and brain memory-forming activity.

Claim 23 (amended) An antibody which binds specifically to an expression product expressed by a host cell comprising an expression vector comprising

~~(a)~~ a DNA molecule comprising the nucleotide sequence of SEQ ID NO:3, operably linked to a promoter~~, or~~

~~(b) a DNA molecule comprising a nucleotide sequence which hybridizes under stringent conditions to the nucleotide sequence of SEQ ID NO:3, operably linked to a promoter~~.

#9
5/16/02

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:

Masato HORIE et al.

Appln. No. 09/787,360 Group Art Unit: 1646

Filed: March 16, 2001 Examiner: Olga N. Chernyshev

For: LY6H GENE

DECLARATION

Honorable Commissioner of Patents and Trademarks

Washington, D.C. 20231

Sir:

I, Masato HORIE, senior researcher, Second Institute of New Drug Discovery, Otsuka Pharmaceutical Co., Ltd., hereby declare that:

1) I am one of the inventors of the instant invention,

2) I graduated from Hokkaido University in 1985 and have been involved in the LY6H project since 1997, and

3) this document is to provide evidence to show the involvement of LY6H in brain memory-forming activity and to provide the results of our measurement, through the use of anti-LY6H antibodies, of the reduced levels of LY6H protein in Alzheimer's patients.

I. Introduction

Alzheimer's disease (AD) is characterized by its selective pathology, affecting the areas of the brain that are primarily involved in cognition and higher-level associative learning while leaving most of the other brain regions intact. The medial temporal lobe, especially the hippocampus and amygdala, of AD patients shows signs of a profound change in physiological functions that accompany the amyloid plaques and neurofibrillary tangles that are hallmarks of the disease.

II. Experiment 1

One important component of the medial temporal system of higher vertebrates involved in the storage of explicit memory is the hippocampus. Thus, we examined the importance of LY6H in the activities of the hippocampus by analyzing the electrophysiological properties of LY6H-knockout mice that we generated. Long-term potentiation (LTP) *in vivo* recording in the hippocampus was performed on mice as described previously (Namgung, U., Valcourt, E., and Routtenberg, A. Brain Res. 689: 85-92, 1995). As shown in figure 1, the LY6H-knockout mice (Ly6h -/-) significantly reduced LTP compared with the control wild-type mice (Ly6h +/+) ($P<0.05$, Schéffe test, n=7 for each group). This indicates that the loss of LY6H expression impairs synaptic transmission in the hippocampus.

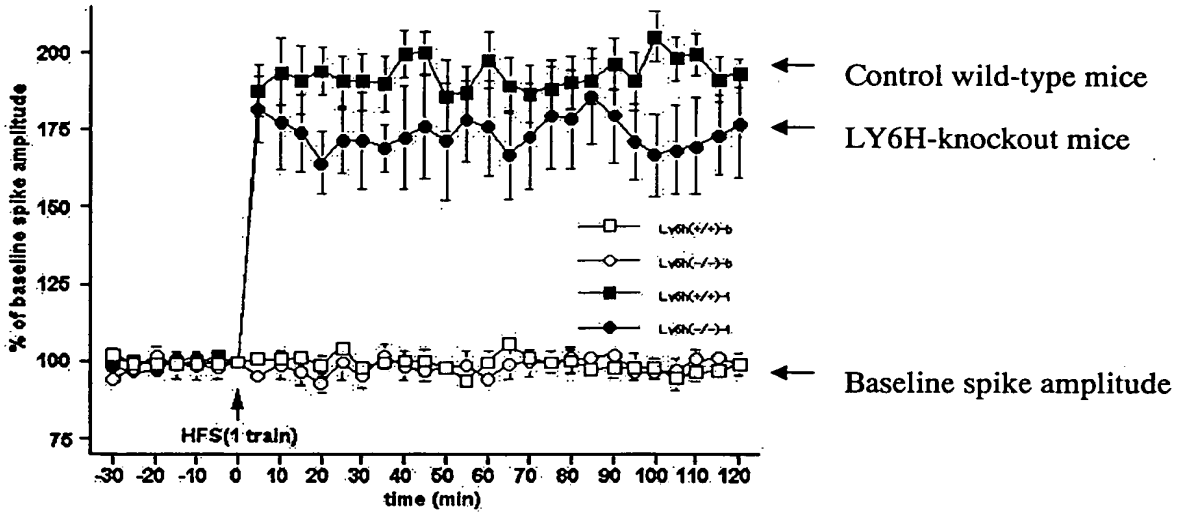


**Fig. 1 Impaired LTP *in vivo* in the LY6H-knockout mouse hippocampus.**

The time course of LTP in the LY6H-knockout (solid circles; n = 7) and the wild-type (solid squares; n = 7) mice is shown for 120 min after tetanus (high frequency stimulation: HFS) at 0 min. Each point represents the mean ± SEM expressed as a percentage of the basal population spike amplitude at 0 min.

III. Experiment 2

The LY6H protein levels in the amygdala of post-mortem brains of AD patients and age-matched non-demented controls were measured by Western blotting with an anti-LY6H antibody. The level of LY6H expression in the amygdala of AD patients is significantly lower than that of the age-

matched non-demented controls (n=9) ($p<0.01$, Student's t-test). The data demonstrate that LY6H is a good diagnostic marker for AD.

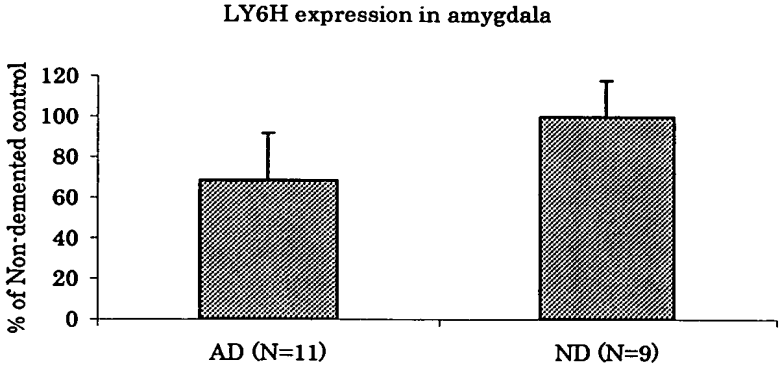


**Fig. 2 Reduced expression of LY6H in Alzheimer's patients.** Figure 2 shows the results wherein bars represent the mean ± SD expressed as a percentage of the LY6H levels of the non-demented controls.

## IV. Conclusion

Although the progressive decline in physiological processes in the brain is the proximate cause of AD dementia, the limitations of biological research methods require static markers to try to reconstruct the physiological changes. According to our findings described above, LY6H will become a key molecule in the field of research and diagnosis of AD.

I, the undersigned, declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date: May 2, 2002

Masato Horie

Masato Horie

ELSEVIER

Brain Research 689 (1995) 85–92

BRAIN RESEARCH

Research report

# Long-term potentiation in vivo in the intact mouse hippocampus

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Accepted 18 April 1995

**Abstract**

We describe the characteristics of long-term potentiation (LTP) in the intact mouse. Perforant path stimulation evokes both a population excitatory postsynaptic potential (pop-EPSP) and a population spike potential (pop-spike) from the hippocampal dentate gyrus in urethane anesthetized animals. LTP, as measured by increased pop-spike amplitude and pop-EPSP slope, was successfully induced and reliably maintained at a stable level for at least 12 h, the longest time tested. The LTP-inducing stimulus (3 trains of 400 Hz, 8 0.4 ms pulses/train) used in two strains of mice was less by half than that used in rat. These parameters for inducing LTP were also successfully applied to obtain LTP in two different transgenic mouse strains: one bearing a F1/GAP-43 promoter-lacZ fusion gene and another which overexpresses the S100$\beta$ gene. We also examined the effects of protein synthesis inhibitors, cycloheximide (CXM) and anisomycin (ANI). When CXM or ANI was given 30 min before LTP induction, there was no persistent loss of LTP at the 4 h time point. However, if CXM was given 4 h before LTP induction, significant decay of the potentiated responses occurred 90 min after induction. Half of the animals receiving CXM but not ANI showed a complete and sudden elimination of the entire response after the LTP-inducing stimulus. It was speculated that loss of a constitutively-expressed housekeeping protein, for example a calcium buffering protein, with an estimated half-life of 2 h would lead to an inability to buffer LTP-induced alterations, such as intracellular calcium elevation, increasing intracellular calcium to toxic levels. LTP can be reliably studied in the intact mouse hippocampus and can be usefully applied in both wild-type and transgenic preparations. It will afford the opportunity to study LTP in mouse mutants in their in vivo state rather than in vitro in the slice preparation permitting characterization of biochemical and molecular events after LTP and then to determine the explicit relation of these events with the physiology of synaptic enhancement.

*Keywords:* Long-term potentiation; Mouse; Dentate granule cell; Protein synthesis; Cycloheximide; Anisomycin; Calcium buffering

## 1. Introduction

As first described in the rabbit, brief tetanic stimulation induces an enhanced, persistent synaptic response, referred to as long-term potentiation (LTP; [2,3,15]). Since then, in vitro studies of LTP using the hippocampal slice preparation in guinea pig [26], rat [31], and mouse [36] have been reported. In spite of its contribution to understanding molecular mechanisms underlying LTP, the in vitro preparation has the limitation of recording duration due to tissue deterioration [32]. Moreover, brain slices are necessarily a deafferented preparation which can alter neuronal physiology [18]. Finally, the deteriorating surfaces may compromise subsequent biochemical analysis [24].

Interest in studying LTP in the mouse has recently arisen as a result of LTP studies of null mutants [12,27]. All of these studies have been carried out in the in vitro slice. Because of concerns listed above, the in vivo study of mouse LTP would provide information not readily available in slice studies. We describe here procedures for stereotaxic placement of electrodes and the methods used to obtain evoked response from mouse dentate granule cells. We have investigated the effect of protein synthesis inhibitors on LTP as had been done in rat [14,20,30] using the protein synthesis inhibitors cycloheximide (CXM) and anisomycin (ANI) in the anesthetized mouse. Finally, we report the presence of potentiation in two transgenic mouse preparations which contain rat F1/GAP-43 promoter-*lacZ* [34] and human S100$\beta$ gene constructs [10].

A portion of the present results appeared in abstract form [19].

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*SDI* 0006-8993(95)00531-5

## 2. Materials and methods

### 2.1. Materials

Mice of the albino ICR, B6D2F1, and B6C3F1 strains (25–35 g) were obtained from Harlan Breeding Farms and C57BL/6 mice were from Jackson Laboratory. Mice were then bred in this laboratory and male offspring were used as subjects. F1/GAP-43-*lacZ* transgenic mice harboring 6 kbp 5′ flanking region along with 11 kbp of the first intron of rat F1/GAP-43 gene connected to 3.5 kbp *E. coli lacZ* gene [34] were kindly provided by Dr. M.C. Fishman. Transgenic offspring were screened by Southern or dot blot hybridization of the tail genomic DNA with $^{32}$P-labeled *lacZ* DNA probe [13]. S100 transgenic mice along with albino CD1 control mice were kindly provided by Dr. J. Roder [10]. CXM and ANI were obtained from Sigma Chemical Co.

### 2.2. Surgery and stimulation / recording procedures

Animals were anesthetized by injecting urethane (1.2 g/kg, i.p.) and placed in the stereotaxic apparatus. It was found that mice required a higher dose of urethane compared to the 0.6 g/kg used in the rat [17]. Since tl anesthetic response was quite sensitive to small differenc in dosage, we therefore injected an initial dose of 1.2 g/l followed by supplemental injections (0.2–0.6 g/kg) needed. In practice when satisfactory planes of anesthes were reached, usually within 1 h, then further doses of 0 g/kg were injected about every 3 h. We used tapered e bars with shorter and blunter tips (tapered at an angle of degrees to the brain surface, tip diameter = 1 mm) th those used for the rat to obtain rigid fixation of the he without middle ear damage. Body temperature was mai tained at 37°C using a heated water jacket pad (Americ Hospital Supply). Brain surface was exposed through ho in the skull. A glass recording electrode with 1–5 μm diameter, back-filled with 0.9% NaCl, was lowered to cell body layer of dentate granule cells (coordinates = mm posterior to bregma, 1.0 mm lateral to the mid-li 1.5–2.0 mm ventral to the brain surface). Bipolar, twis nichrome stimulation electrode (wire tip diameter = μm) was then lowered into the perforant path with angle of 60 degrees to the skull surface (coordinates = mm anterior to lambda, 2.5 mm lateral to mid-line, 1.5– mm ventral to the brain surface). Initial responses w obtained using a cathodal stimulation intensity of 4.0-

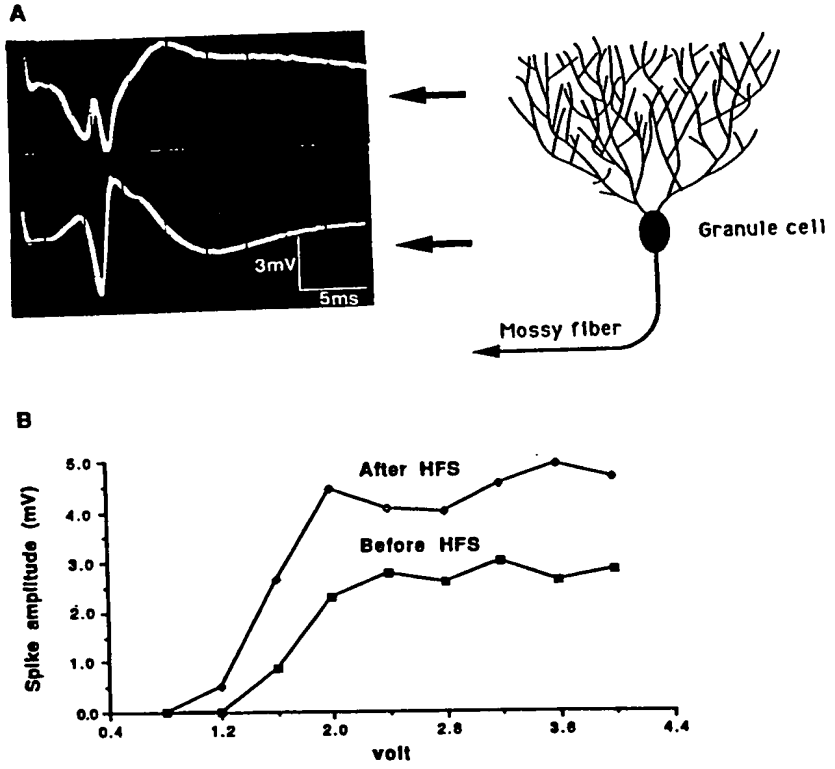


Fig. 1. A: Field potential in mouse dentate gyrus molecular layer (upper) and granule cell layer (bottom) evoked by stimulating the perforant path. trace shows recording from dendritic zone in which the initial negativity, the pop-EPSP represents the sink or inward current flow and the fol positive wave, pop-spike potential, represents the source from the synchronized discharge of granule cells. Lower trace shows the recording at the cel layer in which initial positivity represents the source (the pop-EPSP), followed by inward current representing the pop-spike potential [1,1 Relationship between input stimulation (volt) and output spike amplitude (mV). I/O curve 30 min after LTP induction showed the expected shift

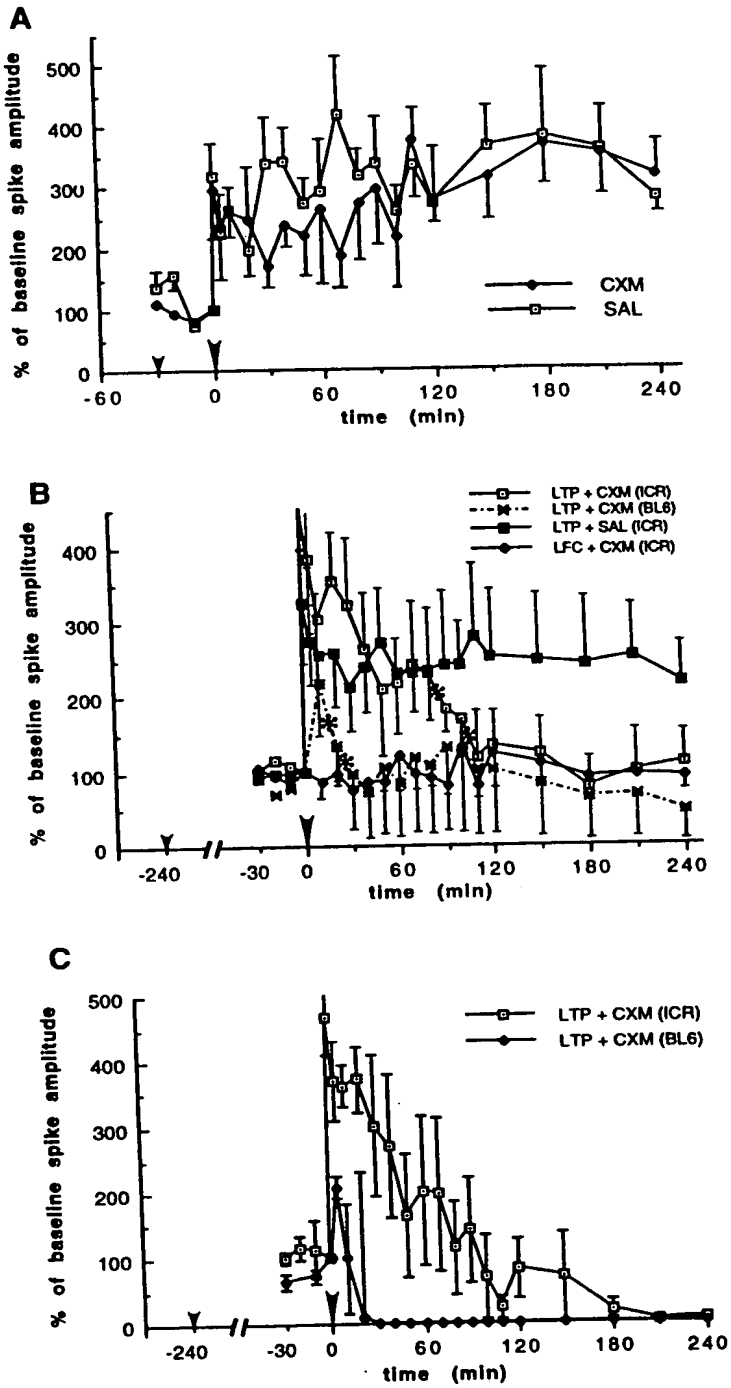


Fig. 3. Treatment of CXM (120 mg/kg) 4 h but not 30 min before LTP induction caused potentiated response to decay. A: CXM or saline (SAL) i
30 min before LTP induction. Note a transient decreased amplitude of CXM-injected group ($n = 3$) for 30–100 min after LTP induction and the re
of the potentiated response compared to controls ($n = 3$). B: CXM injected 4 h before LTP induction. The mean potentiated response of saline-i
control group ($n = 8$) was persistently higher than 200% of baseline spike amplitude for 4 h whereas the mean potentiated response of CXM-inject
mouse group ($n = 8$) began to decay at 90 min after LTP induction (marked by asterisks) and was maintained at the significantly lower level at 1
min after LTP induction. Another mouse strain C57BL/6 ($n = 3$) also showed a decay of the potentiated responses, but at an earlier time point, be
20 min after LTP induction, decaying to baseline at 30 min (marked by asterisks), remaining at the baseline level until the last measurement at 240
Mean response of 4 h CXM-treated ICR ($n = 4$) and C57BL/6 ($n = 2$) mice which displayed a complete loss of response during 4 h recording
Small and large arrowheads in A, B, and C indicate the time points of CXM of saline injection and LTP-inducing stimulus respectively. Vertic
represent S.E.M.

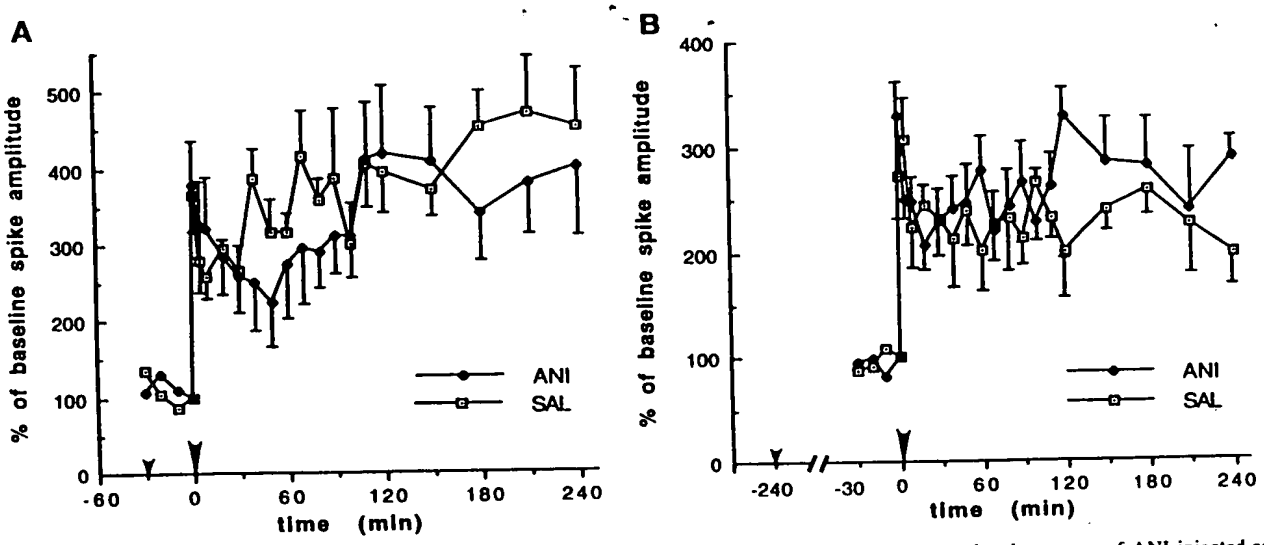


Fig. 4. Anisomycin (ANI) given either 30 min or 4 h before LTP had no effect on its persistence. A: Mean potentiated response of ANI-injected ani ($n = 4$) 30 min before LTP induction compared to saline-injected control (SAL; $n = 4$). Note that, like CXM (Fig. 3A), there was a transient decrea the potentiated response at $t = 40$–90 min. B: Mean potentiated response of ANI- or saline-injected group 4 h before LTP induction ($n = 4$ for each significant difference was found between the two groups during 4 h recording period. Small and large arrowheads in A and B indicate the time poi ANI or saline injection and LTP-inducing stimulus respectively. Vertical lines represent S.E.M.

Effects of 4 h CXM pretreatment was further studied in a dose–response analysis. Mice treated with 90 mg/kg ($n = 4$) and 75 mg/kg ($n = 2$) maintained the potentiated response at 237% ± 9.3% (mean ± S.E.M.) and 254% ± 3.5% respectively during the 100 min recording period without showing a significant difference from saline-LTP control group (% of mean potentiated response = 174.4 ± 9.6; $n = 2$) ($F = 0.54$, df = 2,50, $P > 0.6$). The sudden decay of the potentiated responses, as observed in 120 mg/kg CXM group, was never observed with the lower CXM doses.

### 3.4. Effects of anisomycin (ANI) on LTP

We examined the effects of ANI, another protein synthesis inhibitor on LTP. When ANI was given 30 min before LTP induction, no significant effect on LTP response relative to controls was observed ($F = 0.24$, df = 1,108, $P > 0.5$) though there was a transient decrease in response between 40–90 min ($F = 1.67$, df = 1,36, $P > 0.2$; Fig. 4A). Average responses ($n = 4$) during this period was 362.8% for saline-treated group and 273.5% for ANI treated group. A similar transient decrease, though non-significant, was also observed in a mouse group pretreated with CXM for 30 min before LTP induction (Fig. 3A). Potentiated responses from mice receiving ANI 4 h before LTP showed no significant difference from controls over the 4 h post-LTP recording period ($n = 4$ for both group; $F = 0.48$, df = 1,108, $P > 0.5$; Fig. 4B). The potentiated response of ANI group, though elevated relative to the saline control during the 120–240 min period (mean potentiated responses = 284% and 223% for ANI and saline groups respectively), was not significantly different ( 1.92, df = 1,30, $P > 0.2$).

## 4. Discussion

The present study demonstrates that LTP can be rel studied in the anesthetized mouse preparation. The po ated response measured by baseline spike amplitude usually greater than 200% and no significant deca curred even after 12 h. Its reliability was further confi by the persistent elevation of EPSP slope. This prepa provides a useful alternative to studies in the hippoc slice, and should be particularly helpful in providin sights into the hippocampal chemistry of transgenic p rations after LTP is induced.

In vivo LTP in the mouse has been described i previous reports but with contradictory results. I report, the consequence of tetanic stimulation was t duce a slowly rising increase in response [21]. In co a report in abstract form sets as a criterion for LTP a abrupt increase in the slope of EPSP and the amplit population spike for the 30 min observation period This criterion would rule out the first report as demo ing LTP. The latter authors noted that 20% of norma showed potentiation of EPSP and 64% showed po tion of the spike. In contrast to these reports, we ob a higher success rate and long-lasting, robust potent in our study, 38% (5 of 13) and 77% (10 of 13) of ICR mice showed potentiation of EPSP and the respectively (defined as an increase in EPSP slope o or greater and an increase in spike amplitude of 4

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